Phorid Fly Release and Monitoring Protocols

This document consists of two separate protocols designed as guides to assist installation personnel in releasing *Pseudacteon* spp. flies (common names include ant-decapitating flies, scuttle flies or phorid flies; for simplicity we will use the common name phorid fly in this document) and in follow-up monitoring to determine the fly's establishment and expansion. Phorid fly species in the genus *Pseudacteon* are important natural enemies of imported fire ants (*Solenopsis invicta*, the red imported fire ant; *Solenopsis richteri*, the black imported fire ant; and *Solenopsis invicta* X *Solenopsis richteri*, the hybrid fire ant). The release, establishment and expansion of these phorid fly species is an important sustainable biological control component for Integrated Pest Management (IPM) of fire ants. The ultimate goal is significant reduction in fire ant densities so that reliance on chemical control can be reduced.

Phorid Fly (*Pseudacteon* spp.) Release Protocol

1 . **Determine the fire ant (*Solenopsis invicta*) colony density of the potential release area. Minimum fire ant colony density should be at least 20 colonies per acre (50 colonies per ha.).** As mentioned above, the most important criterion for site selection is sufficient fire ant colony density (~20 colonies per acre or more). Other desired site features of a suitable site include: varied vegetation, cover and proximity to water. Selected sites should also be limited use areas so that routine site management such as insecticide applications (for fire ants and other pests) can be minimized. Early on in phorid fly establishment, exposure of the phorid flies and fire ants to insecticides should be minimal to provide a better opportunity for their survival. Release sites at Camp Robinson and Little Rock Air Force Base exhibited these characteristics; 20 or more colonies per acre, varied vegetation, adequate cover, proximity to water, limited use areas.

Colony density can be easily determined using the ¼ acre circle technique. This is accomplished by use of a ~ 59 ft. rope attached to a tent stake. The tent stake will be tethered to the ground. The evaluator will hold the rope and walk around the area (beginning in the center and moving outward) flagging fire ant colonies and evaluating their activity. After the evaluator has walked over the entire area in a circular manner and is at the far end of the rope, about ¼ acre area has been evaluated. If 25 or more fire ants are visible on the disturbed colony, it is considered active.

Example: If 12 active colonies we found active within the ¼ acre circle, then the colony per acre density is about 48 colonies per acre. This site would have more than adequate colony density to make a phorid fly release.

Fig. 1. Example of estimating IFA colony density using the “1/4 acre circle technique”.

2. Collect imported fire ants for parasitization: Live ants will be collected by disturbing a mound and placing a PVC pipe (~ 11cm diameter x ~28 cm height) on the mound (Fig. 2.1). Ants climb up the pipe and then knocked into a bucket for collection. These ants are then weighed and packaged in 18 x 13 cm lock-lid storage boxes for shipping (Fig. 2.2). The boxes should also contain test tubes filled with water, a cotton ball, and moistened dental plaster to maintain humidity in the boxes. A moist cotton ball should also be provided as a water source. **Colonies from which ants are collected should be marked with a wooden stake and with a GPS unit so that after parasitization, ants can be returned to their original colony. Make certain that colonies and collection containers are accurately marked.**

Fig. 2. Ants climbing onto collection pipe.

Fig. 3. Bucket used to collect ants.

Fig. 4. Snap-lid storage box used for shipping ants.

3. Ship live ants to appropriate agency for parasitization. Use overnight delivery. In the case of the 2013 release at Camp Robinson and Little Rock Air Force Base, the boxes were shipped to the Florida Department of Agriculture in Gainesville, Florida. Installations interested in future phorid fly releases should contact the US Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) to check availability of phorid flies. The appropriate biological control agency lab will either expose (parasitize) your ants to one or multiple phorid fly species. This will often depend upon which phorid fly species already occur in your area and the approximate sizes or your ants. In some situation, collected ants will be exposed in the field rather than a laboratory. After being parasitized, the weights of the exposed (parasitized) ants are normally recorded.

4. Release parasitized ants backs back into their original colony. After exposure, ants will be shipped (overnight) back to you. As soon as possible after receiving the shipment, place the parasitized ants back into their original colony (this is why labeling is so important). On hot dry days the mounds can be spritzed with water before the ants are released. Based on the weights of parasitized ants, it is possible to estimate the number of flies that were released (this data would be supplied by the agency that parasitized the fire ants).

5. Begin monitoring for phorid fly (*Pseudacteon* spp.) presence about 3-4 weeks after parasitized ants are released back into the colony (if possible). Details for phorid fly monitoring are provided below in the “Phorid Fly Monitoring Protocol”.

Phorid Fly (*Pseudacteon* spp.) Monitoring Protocol

Over the last 15 years, researchers and cooperators in federal and state government, educational institutions, and local interest groups have participated in phorid fly releases throughout the fire ant infested regions of the United States. Over the years methods for monitoring phorid flies after release have improved. Use the method that works best for you the observer, and please record what that method is.

When:

- Monitor when conditions are air temp >68°F, clear, and sunny
- The first fly evaluation should be conducted 2 or 3 months after the release. Presence of phorids at this point verifies emergence of the first generation. All adults from the release would have died and development of those eggs to adult should have been completed.
- Annual fly evaluations
  - Evaluation in spring and fall (twice a year) is recommended
  - Often fall monitoring sessions are more successful compared with spring. If only evaluating flies once a year, monitor in the fall.

Where: Initial monitoring should begin at the release site.

- Release site
  - Monitor 2-3 months after release to determine establishment of 1st generation
  - Monitor after first winter to determine overwinter survival
  - Monitor annually for 3 years if no flies found – then we can assume the release was unsuccessful
- Remote sites (sites some distance from the initial release)
  - One year after release: Inspect release site for presence of flies if flies are present then go 2 km from release site in four cardinal directions. If lies are present proceed another 2 km away from release site. If flies not present, close distance to release site by ½ until flies are found.
  - One and one-half years after release: Inspect release site for presence of flies then double distance from previous find (year 1) in four cardinal directions. Continue in same manner as in year one.
  - Two and three years after release: Inspect release site for presence of flies then double distance from previous find (year 1.5, 2, etc.) in four cardinal directions.

How:

- Record site data
  - Release site: date, GPS location, county, species of flies present.
  - Remote site: date, GPS location, county, species of flies present,
- Aspirator method (optional)
  - At each site choose 2-4 active mounds
  - Dig a hole into the mound using a hand towel
  - Disturb the colony by crushing ants in on a shovel then placing the ants back into the colony
  - Aspirate periodically over the mounds using a traditional aspirator for about 10 minutes
  - During high temperatures it may take 20-25 minutes to see the first phorid fly

        - Label vial with location and date and place the closed vials of flies in a small cooler or other insulated container with ice packs or dry ice to chill them
        - Once are immobile or dead, 70-80% ethyl alcohol should be added to the vial to preserve the flies
        - Disadvantage: Often, only the species active during the sampling period will be collected and not all *Pseudacteon* species are active at the same time of day.
    - Puckett et al (2007) trap method modified by Farnum and Loftin (2010)
        - The Puckett sticky trap modified by Farnam and Loftin has been successful in monitoring phorid fly establishment and expansion. This method has a major advantage over aspiration in that the sticky trap can be left out for 24 hours (overnight) to collect phorids. This allows for the collection of multiple species (peak activity of various species often varies with the time of day)
        - At each site place 3-4 traps near a mound (use a shade cover (plastic plates on wire flag)) when ambient temperatures are hot
        - Retrieve traps after 24 hours
        - It is best to place traps during time periods when rain is **not** expected
        - Place sticky part of trap back onto a Styrofoam cup and place in cooler with ice packs to preserve
        - Traps can be baited with dead imported fire ants (500-800 ants or about 1 g) or placed on a disturbed colony (disturbed ants that crawl into the Fluon coated petri dish cannot escape)

Design of the phorid fly trap

Farnum and Loftin modified the Puckett et al (2007) trap as follows

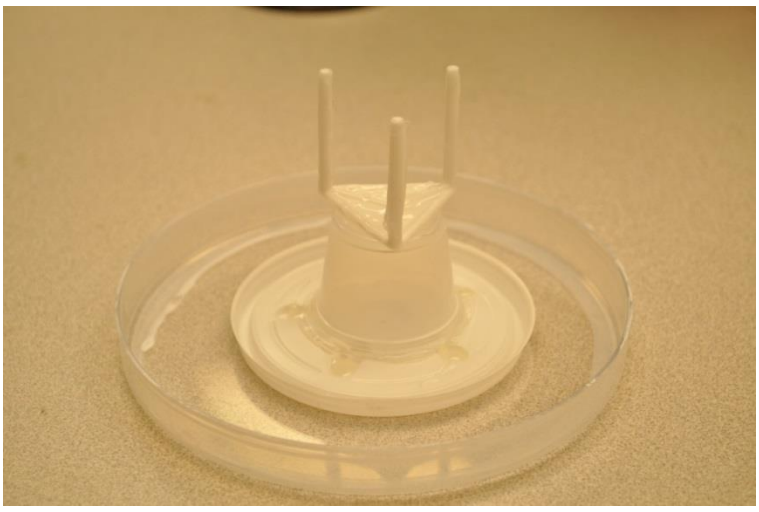
- Application of Fluon coating around lip of 150x15mm Petri dish (uses only one petri dish per trap)
- Pizza tri-stand (Plastic 3-prong pizza stand) is attached to a 1 oz. portion cup with hot glue and the portion cup is attached to a 6 oz. coffee cup lid with hot glue.
- After traps have been assembled (portion cup, pizza tri-stand and coffee cup lid), Fluon coating is applied to the outer surface of the portion cup
- After Fluon has been applied to the portion cup, prongs of the pizza tri-stand are coated in Tanglefoot®
- Traps are baited with 1 g dead imported fire ants (place ants in petri dish) or (optionally) place traps on disturbed fire ant colony
- Once traps are collected (usually after 24 hours), they are easily protected and stored by placing the coffee cup lid onto a Styrofoam coffee cup
- Pizza tri-stands are available at restaurant supply outlets. Coffee cups and lids are readily available at most discount stores.

Identification:

- Keys are available to identify phorid flies, however, a high quality microscope is necessary.
- Phorid fly samples can be shipped to the following address for identification (include all data i.e. date, GPS location, etc.):
    - Kelly Loftin, Cralley-Warren Research Lab, 2601 North Young Avenue, Fayetteville, 72704. Phone: 501 416-3684

Collecting imported fire ants for use as phorid fly bait: Live ants are collected by disturbing a mound and placing a PVC pipe (~ 11cm diameter x ~28 cm height) on the mound. Ants climb up the pipe and then knocked into a bucket for collection. After collection, ants can be killed by freezing, crushing or by microwaving in a microwave oven.

Various phorid fly traps. The traps recommended in this protocol are the live and dead imported fire ant baited Puckett traps (top two images on left)



Picture of Puckett trap modified by Farnum and Loftin.

Key to Identification of *Pseudacteon* Phorid Flies

Ovipositor structure is unique to species among phorids. Use a dissection microscope to examine the posterior end of female flies to determine species (from Porter and Pesquero 2001).

1 Ovipositor simple without lateral lobes, long, curved downward with a large ventral tooth near base; hairs on last abdominal segment not unusually long; Fig. 5…….. ***P. curvatus*** **Borgmeier**

- Ovipositor with lateral lobes in dorsal-posterior view; Figs. 12, 18, 19……**2**

2 Central extension of ovipositor much longer (posteriorly) than the lateral lobes; Lateral lobes rounded and extend out diagonally; central extension cylindrical; Fig 12….***P. litoralis*** **Borgmeier**

- Central extension of ovipositor, at maximum, a little longer than the lateral lobes; Figs. 18, 19 ……..**3**

3 Lateral lobes broadly rounded, each with a membranous extension off their inner borders directed medially; membranous extension in the shape of a stocking; Fig 18 …… ……..……..***P. obtusus*** **Borgmeier**

- Lateral and central lobes pointed, in form of a trident; membranous filament under each lateral lobe; Fig 19……***P. tricuspis*** **Borgmeier**

Ovipositor images for four species of phorid flies. The bar in the corner of each image equals 50 µm.

Figure 5. *P. curvatus* lateral view;
a) from Buenos Aires Province, Argentina
b) from São Paulo State, Brazil

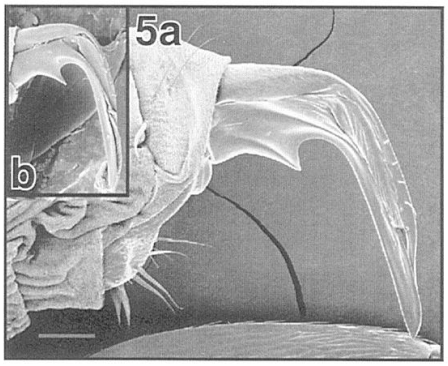


Figure 12. *P. litoralis*
dorsal-posterior view

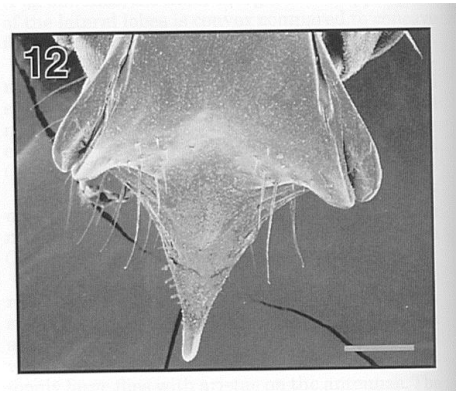


Figure 18. *P. obtusus*
dorsal-posterior view

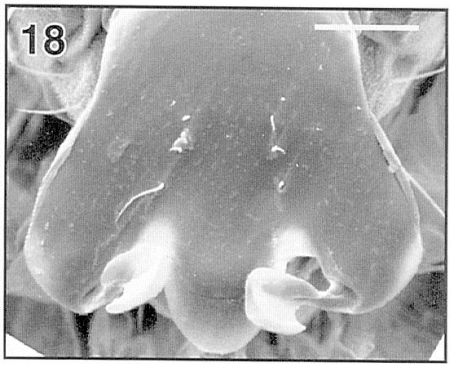
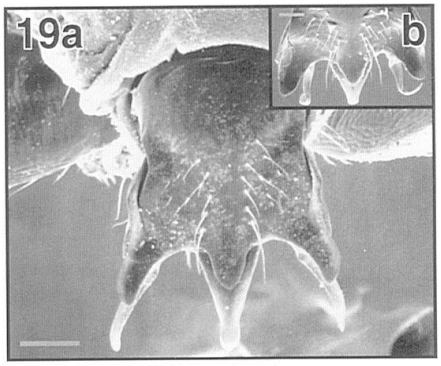


Figure 19. *P. tricuspis* dorsal-posterior view
a) from São Paulo State, Brazil
b) from Buenos Aires Province, Argentina



Key prepared by Dr. Sanford Porter, USDA, ARS-CMAVE, 1600 S.W. 23rd Drive,Gainesville, FL 32608

References

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Puckett, R.T., A. calisto, C.L. Barr and M. Harris. 2007. Sticky traps for monitoring *Pseudacteon* parasitoids of *Solenopsis* fire ants. Environ. Entomol. 36: 584-588.

Trap Placement Protocol (Example from Fall 2014).

Before placing the trap, please use the permanent marker to label the site at which you are placing it. Write the label on the lid of the cup. Place the Petri dish on the ground and put the trap on the dish. Then sprinkle 1 gram of dead ants in the Petri dish around the trap base. (If not using dead ants, place the petri dish on a disturbed mound so that ants will crawl into the petri dish.) Place a flag next to the trap so that I can return to it.

Below is an example code used for labelling phorid fly traps:

Little Rock Air Force Base:

LRAFB MUN: Munitions depot (Place traps along the field where releases took place)

LRAFB SAD: Saddle Club (Place traps across from road from field we used)

LRAFB SAD2: Saddle Club 2nd field (Place traps along the North end of the field)

LRAFB ROW: Right of Way (Handicap hunting plot) (On either side of the road crossing the right of way works here)

Camp Robinson:

CROB AF: Airfield 1st release (along east side of airfield)

CROB AF2: Airfield 2nd release (along west side of airfield)

CROB CARP: CARP Trailhead (5 traps)